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UNIVERSITEIT GENT

FACULTY OF PHARMACEUTICAL SCIENCES

Analytical profiling, modeling and transdermal/transmucosal characteristics of bioactive *N*-alkylamides

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INTRODUCTION

N-alkylamides are a group of bioactive molecules found in several plants. Extracts and formulations derived from these plants are not only used orally, but are applied on the skin and buccal mucosa as well. However, there is no specific information available about the intrinsic local pharmacokinetics of *N*-alkylamides after topical application, questioning the role of this mode of administration. Therefore, we investigated the transdermal and transmucosal behaviour of spilanthol, a prominent *N*-alkylamide, in a commercial *Spilanthes* extract, two mouth gels and different propylene glycol (PG)/H_20 solutions.

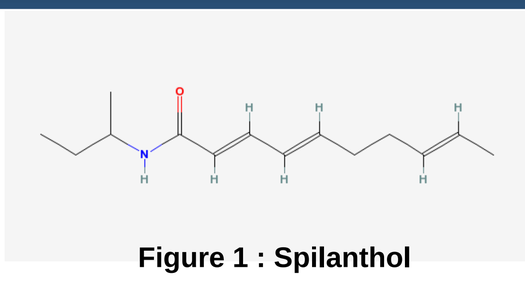

Figure 1 : Spilanthol

EXPERIMENTAL

A HPLC/UV/ESI-MS *N*-alkylamide profiling of a commercial 65% ethanolic *Spilanthes* extract was performed using a prevail RPC_{18} (250 × 4.6 mm, 5 µm) column with an optimized gradient. *In-vitro* penetration studies using static Franz diffusion cells (diffusional area = 0.64 cm²) were performed. The EtOH in the extract was evaporated and the residue was redissolved in different PG aqueous solutions. 500 µL of these liquid formulations and 0.5 g of commercially available mouth gels were applied on dermatomed (~400 µm) human abdominal skin and/or porcine mucosa. The spilanthol content in the FDC receptor fluid was assayed using a isocratic high throughput HPLC-UV method, with a Lichrospher 100 RP C_{18} (150 mm x 2 mm, 5 µm) and Symmetry 100 RP C_{18} (150 mm x 2 mm, 5 µm) column. The mobile phase consisted of 1% formic acid in MeOH/H_2O (72.5 ± 2.5/32.5 ± 2.5, V/V). PBS, PBS + 1% hydroxypropyl-β-cyclodextrine (HPBCD) and EtOH/H_2O (30:70, V/V) were used as receptor phase in the skin experiments, while only PBS + 1% HPBCD was used in the buccal mucosa experiments. To investigate the 100% aqueous delivery, 10 and 30% PG solutions were used as donor phase, with PBS + HPBCD as receptor fluid: extrapolation to 0% PG gave the 100% aqueous delivery.

RESULTS and DISCUSSION

A. LC-MS PROFILING

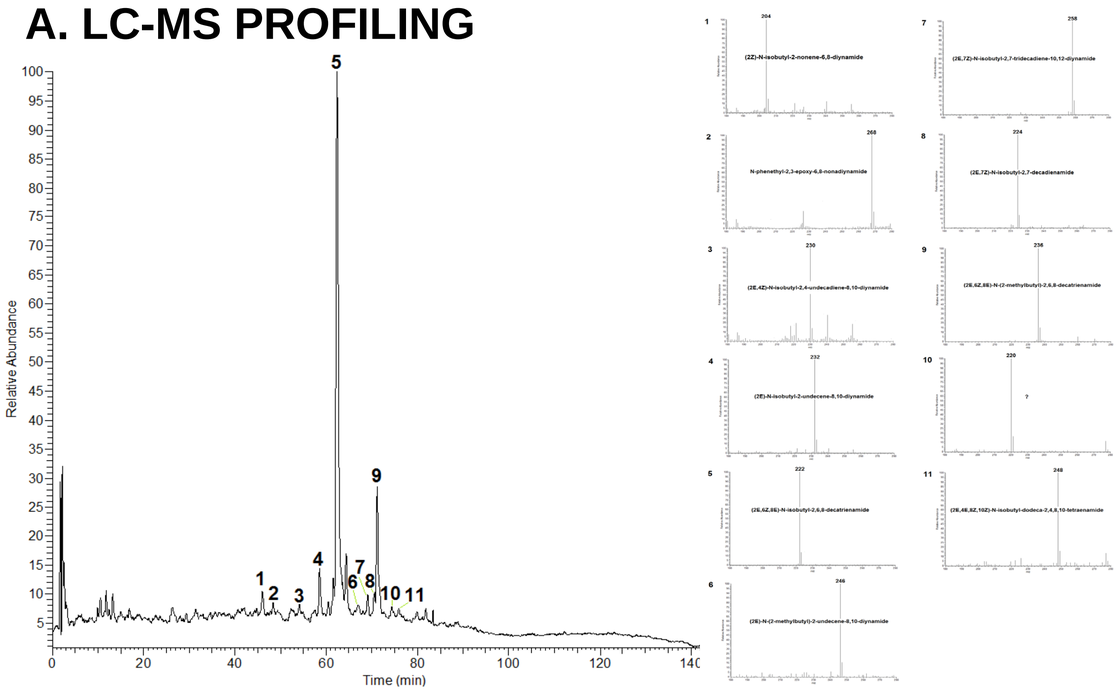

Figure 2 : TIC-MS chromatogram of the identified *N*-alkylamides in ethanolic Spilanthes extract (left) with their MS spectra (right).

Nine *N*-alkylamides structures could be identified, with spilanthol as most abundant alkylamide (± 80%). Two new (*i.e.* not yet identified in ethanolic *Spilanthes* extracts) CID *N*-isobutylamides were detected:

1. <u>compound 8</u> (at 70.5 min): based upon the fragment ions, the alkyl portion for the *m/z*=224 isobutylamide is suggested to be a $C_{10}H_{15}$ diene structure and has a structure formula of $C_{14}H_{25}NO$.
2. <u>compound 10</u> (at 74.3 min): the isobutylamide with *m/z*=220 possesses a structure formula of $C_{14}H_{21}NO$, with an alkyl chain probably being a tetraene.

Further structure elucidation of these two new *N*-isobutylamides is on-going.

B. TRANSDERMAL BEHAVIOUR

We demonstrated that spilanthol permeates the human skin. Partitioning was strongly dependent upon the donor-vehicle composition, while diffusion was mainly influenced by the receptor fluid composition. Human skin $K_{p,aq}$ (± SEM) is 3.31 (± 0.28) · 10^{-3} cm/h [1].

C. TRANSMUCOSAL BEHAVIOUR

For the first time, it is demonstrated that spilanthol permeates mucosa (Fig. 3). Gel 1 (Indolphar®) is adequate to achieve local effects, while gel 2 (Buccaldol®) realizes more systemic effects. A solvent influence is seen with a two times lower permeability for spilanthol in the 65% ethanolic extract compared to 10 and 30% PG based extracts. No influence of the percentage PG (in a range of 10 to 30%) was observed (Fig. 4).
Porcine buccal mucosa $K_{p,aq}$ (± SEM) = 11.293 (± 0.403) · 10^{-3} cm/h.

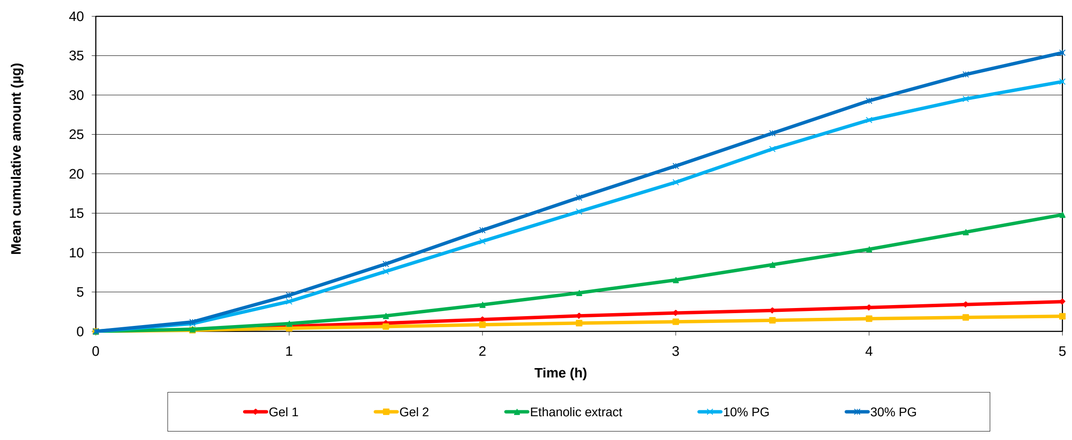

Figure 3: Mean cumulative amount vs. time curves for spilanthol using different dose solutions.

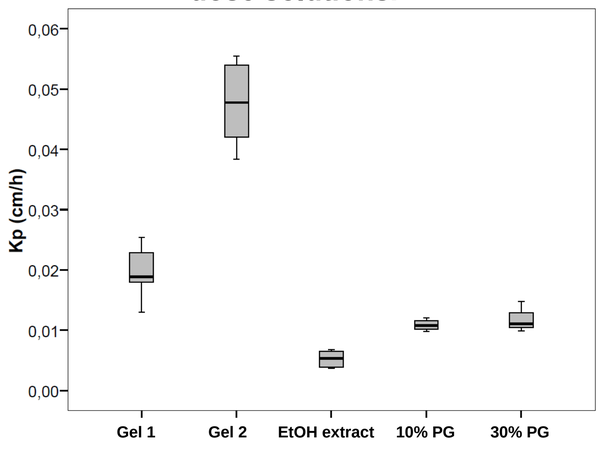

Figure 4: K_p of spilanthol for the different formulations.

CONCLUSIONS

Using HPLC-ESI-MS, 11 *N*-alkylamides were detected in ethanolic *Spilanthes* extract: eight *N*-isobutylamides, two 2-methylbutylamides and one 2-phenylethylamide. Two new N-isobutylamides were identified. Moreover, we demonstrated and quantified that spilanthol can permeate both skin and mucosa in a formulation dependent way.

REFERENCES

[1] J. Boonen, B. Baert, N. Roche, C. Burvenich and B. De Spiegeleer. Transdermal behaviour of the N-alkylamide spilanthol (affinin) from *Spilanthes acmella* (Compositae) extracts, J. Ethno-Pharmacology (2009), doi: 10.1016/j.jep.2009.09.046